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The impact of 27-hydroxycholesterol, a macrophage-synthesized estrogen receptor agonist, on breast cancer pathophysiology.

PRINCIPAL INVESTIGATOR:
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our lab has recently demonstrated that 27-hydroxycholoesterol (27HC) acts as an estrogen. Macrophages have been shown to be a major source of 27HC, and macrophage infiltration is associated with more aggressive tumors. Therefore, the goal of this study is to determine the role of 27HC and macrophages in breast cancer pathology. In this reporting period we have initiated a breeding scheme to establish mice that carry the PyMT transgene with the following genotypes: CYP27A1+/+, CYP27A1-/-, CYP7B1+/+, CYP7B1-/-. We now have breeding mice from 3 out of 4 of these lines. Preliminary data from injection studies reveal that 27HC increases primary tumor growth, increases growth of total tumor burden and decreases the time to secondary tumor detection in PyMT mice. We have optimized conditions to differentiate macrophages. We have found that macrophages secrete factors that increase breast cancer cell proliferation, and provide evidence that one of these factors is likely to be 27HC. Furthermore, the proliferative factors secreted by macrophages appear to act on breast cancer cells in an ER dependant way. Finally, we have shown that a CYP27A1 inhibitor reduces the capacity of macrophages to promote breast cancer proliferation. In conclusion, our data thus far provides strong support for our hypothesis that macrophage secreted 27HC directly impacts the breast tumor environment.

15. SUBJECT TERMS

27-hydroxycholesterol, Estrogen receptor (alpha), Mammary, Tumor associated macrophage, Cytochrome P450 enzyme 27 A1, Cytochrome P450 enzyme 7 B1, Polyoma middle T.

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Title: The impact of 27-hydroxycholesterol, a macrophage-synthesized estrogen receptor agonist, on breast cancer pathophysiology.

Introduction:

The most recent estimates indicate that the lifetime risk of getting breast cancer in women is around 1 in 10 (National Cancer Institute). Significant progress has been made in the development of antihormonal/hormone ablative strategies for the treatment of estrogen receptor positive (ERα+) breast cancers including the aromatase inhibitors (AIs) and Selective Estrogen Receptor Modulators (SERMs) such as tamoxifen. Despite their success in the adjuvant setting, in the metastatic setting most ERα+ tumors respond initially to anti-hormonal therapy but resistance arises and patients are required to endure more morbid cytotoxic therapies (1). Thus, there is a clear need for studies which will lead to the development of new chemopreventative strategies and lifestyle changes that reduce cancer incidence. The mechanisms underlying acquired resistance remain unclear, and appear to be multi-factorial (for review see: 1). One of the most perplexing issues that has yet to be resolved is how tumors become resistant to AIs when the enzyme is intact and when circulating levels of estradiol remain undetectable. It is within the context of this observation, and the general problem of resistance to anti-hormone therapy as a whole, that we propose the hypothesis that breast tumors may be able to produce an a-typical estrogenic ligand whose synthesis is not impacted by current interventions. The recent demonstration that the oxysterol 27-hydroxycholesterol (27HC) functions as a SERM in animal models of atherosclerosis and in cellular models of breast cancer was therefore intriguing (2). Macrophages have been shown to be a major source of 27HC, and it is well established that tumor associated macrophages (TAMs) are associated with more aggressive tumors and worse patient outcome (for review see: 3). Therefore, the major goal of this study is to determine the role of 27HC and TAMs in breast cancer pathology. The central hypothesis which is being addressed is that macrophages contribute to ERα+ breast cancer pathology by producing or secreting 27HC and impacting the local tumor microenvironment.

Body: *(Months 1-12)*

Specific Aim 1: The role of 27HC in the pathophysiology of breast cancer *in vivo*.

As stated in the grant proposal, it was necessary to establish a model system that allows for the evaluation of tumor associated macrophages in breast cancer. To accomplish this, we have chosen to use a mouse model of mammary carcinoma (polyoma middle T, PyMT) (4). These mice carry the MMTV-PyVT transgene, develop spontaneous adenocarcinomas of mammary epithelium by 8 weeks of age, and have been validated as an appropriate model to investigate the multistep progression of breast cancer, are estrogen responsive and readily metastasize to the lung (5-7). Two complementary experiments are being used to address our main hypothesis: (A) Test whether the products of the enzymes CYP27A1 or CYP7B1 in TAMs have an effect on tumor pathogenesis, and (B) define the specific role of 27HC in tumor pathogenesis. We received IACUC and ACURO approval for all of our intended animal studies **(Milestone 1)**.

(A)

The studies proposed in the first experiment required us to establish a breeding program to generate mouse models where either the gene that synthesizes 27HC (CYP27A1) or the gene responsible for its metabolism (CYP7B1) has been knocked out. Since CYP27A1-/- mice and CYP7B1-/- are maintained on different strains, we had to generate four new mouse models: (1) CYP27A1+/+, PyMT+, (2) CYP27A1-/-, PyMT+, (3) CYP7B1+/+, PyMT+, and (4) CYP7B1-/-, PyMT+. We obtained 5 male PyMT+ mice from Jackson Labs **(Task 1)**, and implemented a breeding scheme to obtain mice with the required genotypes **(Figure 1)**. We have now optimized DNA genotyping protocols and initiated our breeding scheme **(Task 2)**. In fact we have recently bred our first males of the following genotypes: (1) CYP27A1+/+, PyMT+, (2) CYP27A1-/-, PyMT+, and (3) CYP7B1-/-, PyMT+ **(Task 2)**. We expect to have CYP7B1+/+, PyMT+ males within the next couple of months. Since we have successfully established CYP7B1-/-, PyMT+, we have now established our first experimental CYP7B1-/-, PyMT+ females. Several have now developed tumors which we currently monitoring **(Task 3)**. Therefore, we are on schedule with meeting the objectives of this experiment.

(B)

The second experiment contained within Specific Aim 1 was designed to directly implicate 27HC or its metabolites in breast cancer pathology. We obtained 5 male PyMT+ (otherwise wildtype) mice from Jackson Labs **(Task 1)**. A program where PyMT+ males are bred to PyMT- females has been established to generate PyMT+ females for use in injection studies **(Task 2)**. In order to eliminate the confounding effects of estrogen, we have used ovariectomized females for our experiments. Injection studies with placebo and 27HC are now underway **(Task 3)**. Interestingly, our preliminary data indicate that primary tumors grown in mice treated with 27HC appear to grow faster than those grown in placebo treated mice **(Figure 2)**. However, at a certain point, this increased growth rate levels off to be similar or slower than placebo treated mice. Furthermore, 27HC treatment decreases the time period from the onset of the primary tumor to the detection of a second palpable tumor **(Figure 3)**. Based on our results, shown in **Figure 2**, this decreased time is likely due to a 27HC mediated increase in the growth rate of secondary tumors. Indeed, we have found that 27HC treated mice have an increased total tumor burden **(Figure 4)**. In order to achieve statistical significance, we are currently breeding additional mice to increase our N values in these experiments. Collectively, our preliminary data lends strong support to our hypothesis that 27HC impacts the growth of mammary tumors.

We are intrigued by the observation that 27HC increases the initial growth rate of mammary tumors but its presence appears to slow growth of established tumors. This has led us to ask whether these tumors lose ER expression at the “inflexion point”, or if the phenotype could be explained by actions of 27HC on another target ie LXR. Indeed we and others have shown that 27HC can activate the liver X receptors. Therefore, in order to provide the proper context to address this question, we need to establish what effects of 27HC are mediated by the ER. In order to achieve this, we will examine the effects of a pure ER ligand, estradiol, on tumor growth in these animals. To this end, we have started breeding animals to be treated with estradiol. Their tumor growth curves will be directly compared to those of placebo and 27HC treated mice.

In summary, Specific Aim 1 is well underway and yielding exciting preliminary results which support our hypothesis that 27HC impacts mammary tumors.

Specific Aim 2: 27HC mechanism of action in breast cancer pathology.

In the original grant proposal we hypothesized that 27HC secreted by macrophages may act in a paracrine manner to affect breast cancer cell biology, or in an autocrine manner by inducing the synthesis of additional macrophage secreted factors (such as SDF-1 and TNFα) which then act on breast cancer cells to increase proliferation and/or invasiveness. We have performed a series of *in vitro* experiments to test this model.

It has become clear that 27HC can act directly on ERα+ breast cancer cells (**Figure 7** and (8)) What was less clear is whether macrophages synthesize 27HC in sufficient quantity to impact breast cancer biology. Therefore, have validated and optimized methods to differentiate primary bone marrow derived monocytes into macrophages **(Experiment 2.1-Task 1)**. After extraction, whole bone marrow is cultured in the presence of recombinant macrophage colony stimulating factor (M-CSF). We have followed a marker of macrophage maturation (CD68) throughout our culture period and found that it obtains maximal expression between day 6 and 8 **(Figure 5)**. Therefore, we will conduct our future experiments after 8 days of differentiation. To add robustness to our studies we have also validated the RAW264.7 and human THP-1 macrophage models (data not shown).

As expected, estradiol treatment increases proliferation of ERα+ human MCF7 breast cancer cells compared to vehicle treated cells **(Figure 6a)**. 27HC also increases the proliferation of these cells **(Figure 7)**. This stimulatory effect of E2 and 27HC could be inhibited by co-treatment with a pure ER antagonist (ICI 182780, ICI) (**Figure 6a,** and data not shown). Thus, the proliferative effects of E2 and 27HC are ER mediated **(Experiment 2.2-Tasks 2, 3)**.

In order to determine whether macrophages could synthesize sufficient 27HC to impact MCF7 cell proliferation **(Experiment 2.1-Task 2)**, we made use of macrophages derived from wildtype (CYP27A1+/+) or from mice that lack the capacity to synthesize 27HC (CYP27A1-/-). Spent media from these macrophages was added to MCF7 breast cancer cells and proliferation was followed through time. Spent media from wildtype macrophages significantly increased MCF7 cell proliferation above cells grown in basal media as well as cells treated with estradiol **(Figure 6a, b)**. Importantly, spent media obtained from macrophages lacking the enzyme responsible for synthesis of 27HC (CYP27A1-/-) had reduced proliferation compared to the wildtype control, although they still had increased proliferation compared to treatment with basal media **(Figure 6b)**. Therefore, macrophages secrete factors which enhance MCF7 cell proliferation, one of these factors being 27HC. Interestingly, co-treating cells with an ER antagonist (ICI) attenuated the proliferative effect of spent macrophage media to near basal levels **(Figure 6b)**. This indicates that in addition to 27HC, the proliferative effects of secreted macrophages are also ER dependant **(Experiment 2.2-Task 2)**.

Spent media from wildtype macrophages or macrophages that lack the enzyme to metabolize 27HC (CYP7B1-/-) both resulted in similar increased MCF7 proliferation rates **(Figure 6c, Experiment 2.1-Task 2)**. Due to their similar effects on MCF7 proliferation, we conclude that either 1) the cells are already proliferating to a maximum extent, 2) 27HC levels do not increase significantly compared to wildtype levels over our time-period, or 3) this enzyme does not play a significant role in macrophages. In the coming year, we will probe the precise mechanisms behind this observation.

We have recently obtained the CYP27A1 inhibitor, G267X, from our collaborators at GlaxoSmithKline. In preliminary experiments, treatment of MCF7 cells with only G267X did

not significantly alter proliferation rate compared to the vehicle control, while both estradiol and 27HC increased proliferation **(Figure 7)**. As previously demonstrated, spent media from wildtype macrophages significantly increased the proliferation rate. However, when macrophages are pretreated with G267X and this media is used to treat MCF7 cells, the proliferation rate is retarded. Therefore, by inhibiting the macrophage synthesis of 27HC, we could reduce the proliferation rate of breast cancer cells, lending strong support to our hypothesis **(Experiment 2.1-Task 2)**.

Collectively, data obtained from these experiments indicates that macrophages produce 27HC which can then act in a paracrine manner on breast cancer cells to stimulate proliferation in an ER dependant fashion. In the coming year, we will investigate possible autocrine actions of 27HC which may lead to the secretion of additional factors that impact breast cancer biology.

Key Research Accomplishments:

In the past year we have completed several *in vitro* studies implicating macrophage-secreted 27HC in breast cancer pathology, and are in the process of developing animal models to study the impact of 27HC *in vivo*.

- We have established a breeding colony of mice carrying the PyMT transgene.
- Crossbreeding has resulted in the development of the following mouse models:
    - CYP27A1+/+, PyMT+
    - CYP27A1-/-, PyMT+
    - CYP7B1-/-, PyMT+
- Preliminary data from injection studies reveal that 27HC increases primary tumor growth, increases growth of total tumor burden and decreases the time to secondary tumor detection.
- Optimized culture conditions for the differentiation of primary macrophages.
- Macrophages secrete factors that increase breast cancer cell proliferation.
- One of these factors is likely 27HC.
- The proliferative factors secreted by macrophages appear to act in an ER dependent way.
- The CYP27A1 inhibitor, G267X reduces the capacity of macrophages to promote breast cancer proliferation.

Reportable Outcomes:

- Crossbreeding has resulted in the development of the following mouse models:
    - CYP27A1+/+, PyMT+
    - CYP27A1-/-, PyMT+
    - CYP7B1-/-, PyMT+
- Presented abstract at 2010 Keystone Symposia: Nuclear Receptors: Signaling, Gene Regulation and Cancer. Abstract 262, “27-Hydroxycholesterol, and endogenous SERM and LXR agonist negatively impacts bone formation in mice.”

Conclusion:

Our current research is directed towards an evaluation of the potential impact of 27HC secreted by tissue associated macrophages in breast cancer pathology. Both *in vitro* and *in vivo*

approaches are being employed to determine specific versus tumor microenvironment effects. Our primary hypothesis is that that macrophages contribute to ERα+ breast cancer pathology by producing or secreting 27HC and impacting the local tumor microenvironment. We have generated three of four genetic mouse models that we will use to test this hypothesis. Using PyMT mice that spontaneously develop breast cancer, our preliminary data shows that elevating 27HC by daily injection results in a primary tumor that grows faster than in placebo treated mice. The time to detection of a secondary tumor is reduced in 27HC treated mice. Furthermore, the total tumor burden (summed volume of all palpable tumors) is increased in 27HC treated mice. Our *in vitro* studies provide evidence that 27HC, which is synthesized and secreted from macrophages can significantly enhance breast cancer cell proliferation. Importantly, we have shown that when macrophages are treated with a CYP27A1 inhibitor, their secretome has a reduced capacity to stimulate breast cancer proliferation. In conclusion, our data thus far provides strong support for our hypothesis that macrophage secreted 27HC directly impacts the breast tumor environment.

References:

1. **Hurvitz SA, Pietras RJ** 2008 Rational management of endocrine resistance in breast cancer: a comprehensive review of estrogen receptor biology, treatment options, and future directions. Cancer 113:2385-2397
2. **Umetani M, Domoto H, Gormley AK, Yuhanna IS, Cummins CL, Javitt NB, Korach KS, Shaul PW, Mangelsdorf DJ** 2007 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. Nat Med 13:1185-1192
3. **Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, Rimoldi M, Biswas SK, Allavena P, Mantovani A** 2008 Macrophage polarization in tumour progression. Semin Cancer Biol 18:349-355
4. **Guy CT, Cardiff RD, Muller WJ** 1992 Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. Mol Cell Biol 12:954-961
5. **Dabrosin C, Palmer K, Muller WJ, Gauldie J** 2003 Estradiol promotes growth and angiogenesis in polyoma middle T transgenic mouse mammary tumor explants. Breast Cancer Res Treat 78:1-6
6. **Maglione JE, Moghanaki D, Young LJ, Manner CK, Ellies LG, Joseph SO, Nicholson B, Cardiff RD, MacLeod CL** 2001 Transgenic Polyoma middle-T mice model premalignant mammary disease. Cancer Res 61:8298-8305
7. **Qin L, Liao L, Redmond A, Young L, Yuan Y, Chen H, O'Malley BW, Xu J** 2008 The AIB1 oncogene promotes breast cancer metastasis by activation of PEA3-mediated matrix metalloproteinase 2 (MMP2) and MMP9 expression. Mol Cell Biol 28:5937-5950
8. **Dusell CD, McDonnell DP** 2008 27-Hydroxycholesterol: a potential endogenous regulator of estrogen receptor signaling. Trends Pharmacol Sci

Effects of estrogen and epidermal growth factor on the ...tion of *PHLDA1* expression in the MCF-7 breast cancer cells

...esa Garcia, SA and Nagai, MA
...tina de Oncologia, Depto. de Radiologia da Fac. de Medicina da ...rsidade de São Paulo, São Paulo, Brazil. 01296-903; nagai@usp.br

...A1 (pleckstrin homology-like domain, family A, member 1; also named ...351) is located on chromosome 12q15 and encodes a 262 amino acid ...in that is a member of the pleckstrin homology-related domain family. ...A1 expression is induced by a variety of external stimuli and there are ...ces showing that it might act as a mediator of anoikis and senescence. ...*A1* has been previously identified by our group as a potential target of ...rogen receptor (ER) action in breast cancer cells. In the present study ...estigated the effects of the E2 (17β- estradiol) and EGF (Epidermal ...h Factor) on *PHLDA1* transcriptional regulation in MCF-7 breast cancer ...ing quantitative Real Time PCR analysis. MCF-7 cells growing in ...d serum were treated with E2 (10^{-8}M) and EGF (50 ng/ml) alone or in ...sence of ICI 182,780 (ICI, 1μM) for 2, 6 and 24 h. The expression of ...*A1* increased 2,25-fold after E2 treatment for 6h and 2,8-fold after EGF ...nt for 2h. MCF-7 cells exposed to E2 after the pre-treatment with ICI ...d PHLDA1 expression similar to that observed after the ICI treatments ...Pretreatment with ICI do not modified the effect of EGF on PHLDA1 ...sion in MCF-7 cells. Exposure of the MCF-7 cells with 5μM of the PI- ...bitor (LY294002), p38MAPK inhibitor (SB202190) or pERK1/2 inhibitor ...059) affects PHLDA1 transcriptional regulation by EGF. Both E2 and ...creased ER phosphorylation on the critical residue serine 118. These ...s suggest that *PHLDA1* mRNA expression is modulated by E2 *via* ...d by EGF through the activation of different cell signaling pathways. ...er, further studies are required to determine if there is a cross-talk ...n ER and EGFR signaling pathways on the regulation of *PHLDA1* ...sion in breast cancer cells. Supported by FAPESP and CNPq.

262 **27-Hydroxycholesterol, an endogenous SERM and LXR agonist, negatively impacts bone formation in mice**

Nelson ER, DuSell CD, Wang X, Michalek RD, Abdo J, Moedder UL, Umetani M, Gesty-Palmer D, Javitt NB, Rathmell JC, Khosla S, and McDonnell DP.
Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, 27707.

Osteoporosis and age related decline in bone mass are important public health concerns, with an estimated 55% of Americans over the age of 50 at risk. 27-Hydroxycholesterol (27HC), a primary metabolite of cholesterol, has recently been characterized as an endogenous selective estrogen receptor modulator (SERM). Thus, given the protective role of estrogens in bone, it was of interest to examine the impact of this oxysterol on skeletal integrity. In addition, we and others have determined that 27HC is a Liver X Receptor (LXR) agonist, although the impact of this pharmacological activity on bone biology is unclear. Further underscoring the need to understand the impact of 27HC on bone are data that reveal an association between metabolic disease and osteoporosis, and studies that report improved BMD in statin treated patients. Cumulatively, these data prompted us to undertake a comprehensive analysis of the impact of manipulating 27HC levels on bone biology in mice. We observed decreased bone mineral density and related changes in bone micro-architecture in mice that exhibit elevated 27HC as a result of (a) the genetic elimination of CYP7B1, the enzyme responsible for catabolism of this oxysterol, or (b) following the administration of exogenous 27HC. Surprisingly, estrogen supplementation only partially reversed the effect of 27HC implicating the involvement of a target other than the ERs. It was of interest therefore, that in cultured primary osteoblasts, 27HC, acting through the LXRs leads to increased TNFα production resulting in a commensurate increase in RANKL, a mediator of osteoclastogenesis. Using co-culture assays we determined that the 27HC dependent production of RANKL in osteoblasts leads to increased osteoclastogenesis. Furthermore, we also demonstrate that 27HC, (a) decreases preosteoblast proliferation, (b) represses the expression of genes associated with osteoblast differentiation, and (c) decreases osteoblast activity. Therefore, by both decreasing bone deposition and increasing bone resorption, 27HC has unfavorable effects on bone. These data establish a firm association between 27HC and bone quality, a conclusion that has far-reaching medical implications.
NIHR37DK48807 (DPM), DOD postdoctoral award BC085585 (ERN).

Androgen receptor activity and prostate specific antigen – a non ...sociation

...Nenonen[1], Paal-André Skjaerpe[2], Aleksander Giwercman[1], Johan ...g[2] and Yvonne Lundberg Giwercman[1]
...ment of Clinical Sciences, Lund University, Malmö, Sweden
... of Clinical Medicine, University of Tromsø, Tromsø, Norway

...rogen receptor (AR) contains a polymorphic CAG segment, which has ...sumed to be inversely associated with AR function. However, analyses ...n data based on this theory have shown conflicting results regarding ...ciation between CAG number and phenotypic characteristics, such as ...cancer.
...vious *in vitro* studies mostly have been based on extreme CAG lengths ...ter-systems containing viral promoters, the aim of this study was to ...e the impact of short, median length and long CAG repeats, but still ... normal range, on the AR activity *in vitro* and *in vivo*.
... >30 experiments on CAG lengths 16, 22 and 28 in reporter-assays ...uman prostate specific antigen (PSA) promoter as target, we found ...edian length (CAG22) was the most efficient compared to CAG16 and ...20% and 12%, respectively thereof; p<0.05 for both). Since PSA, which ...used in the screening and follow-up of men treated for prostate cancer, ...ed by androgens, this finding prompted us to reanalyze available data ... serum PSA concentrations and CAG repeat number in 199 Swedish ...nt men and also to include a cohort consisting of 172 elderly men from ...e study in Norway. These men had no priori diagnosis of benign or ... prostate disease.
... 40% and 150% higher PSA concentrations in the CAG22 group as ... to those with CAG>22, in adolescent and elderly men, respectively. ...ysis of CAG repeat lengths in linear regression models, as performed ...udies, probably is not valid and, at least in some tissues, the median ...y be associated with the most efficient AR. From a clinical point of view, ...e further investigated whether the cut off levels used in PSA screening ...n for additional diagnostic procedures should be individualized according ...notype.

... Swedish Research Council (grant K2009-54X-21116-01-3), the Swedish ...ociety (grant 08 0351 and 07 0139), the Research Fund and Cancer ... Fund of Malmö University Hospital, and the Gunnar Nilsson Cancer ...on.

264 **Insights into Estrogen and SERM activity in breast and uterus revealed by genome-wide expression and ChIP-chip analysis**

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Department of Biochemistry, University of Wisconsin, Madison, WI, 53706

17-β Estradiol (E2) increases the rate of proliferation of certain tumors and is antagonized by selective estrogen receptor modulators (SERMs). Our previous work has centered on E2 action and the antagonistic properties of SERMs in MCF-7 cells. Our current studies focus on the effects of E2 and selected SERMs on the ECC-1 endometrial adenocarcinoma cell line. Binding sites for ERα, RNA PolII, and AcH4 were identified using genome-wide ChIP-chip and revealed a distinctly different set of target genes from those found in MCF-7 cells. Upon E2 treatment, ERα was recruited to sites containing canonical EREs, often at significant distances from promoter regions, and initiated changes in PolII and AcH4 occupancy at neighboring genes. A large majority of these genes were confirmed as E2 targets by genome-wide expression analysis. Regulated genes were known to play roles in angiogenesis, cell growth and survival, and tissue development and morphogenesis. Importantly, a select class of E2 targets was equivalently regulated by 4-OH-Tamoxifen (TAM) and to a lesser extent by Raloxifene (RAL). TAM also increased the expression of several genes that were not regulated by E2, including cancer-specific antigens known to elicit immune responses. The strong agonist activity of TAM is largely limited to upregulated genes and is consistent with clinical data demonstrating significant effects of TAM treatment on the uterus. In contrast, neither SERM exhibited any partial agonist activity in the MCF-7 cell line. These studies establish the ERα cistrome in ECC-1 cells and provide insight at a genomic level into the tissue-selective nature of estrogens and SERMs.

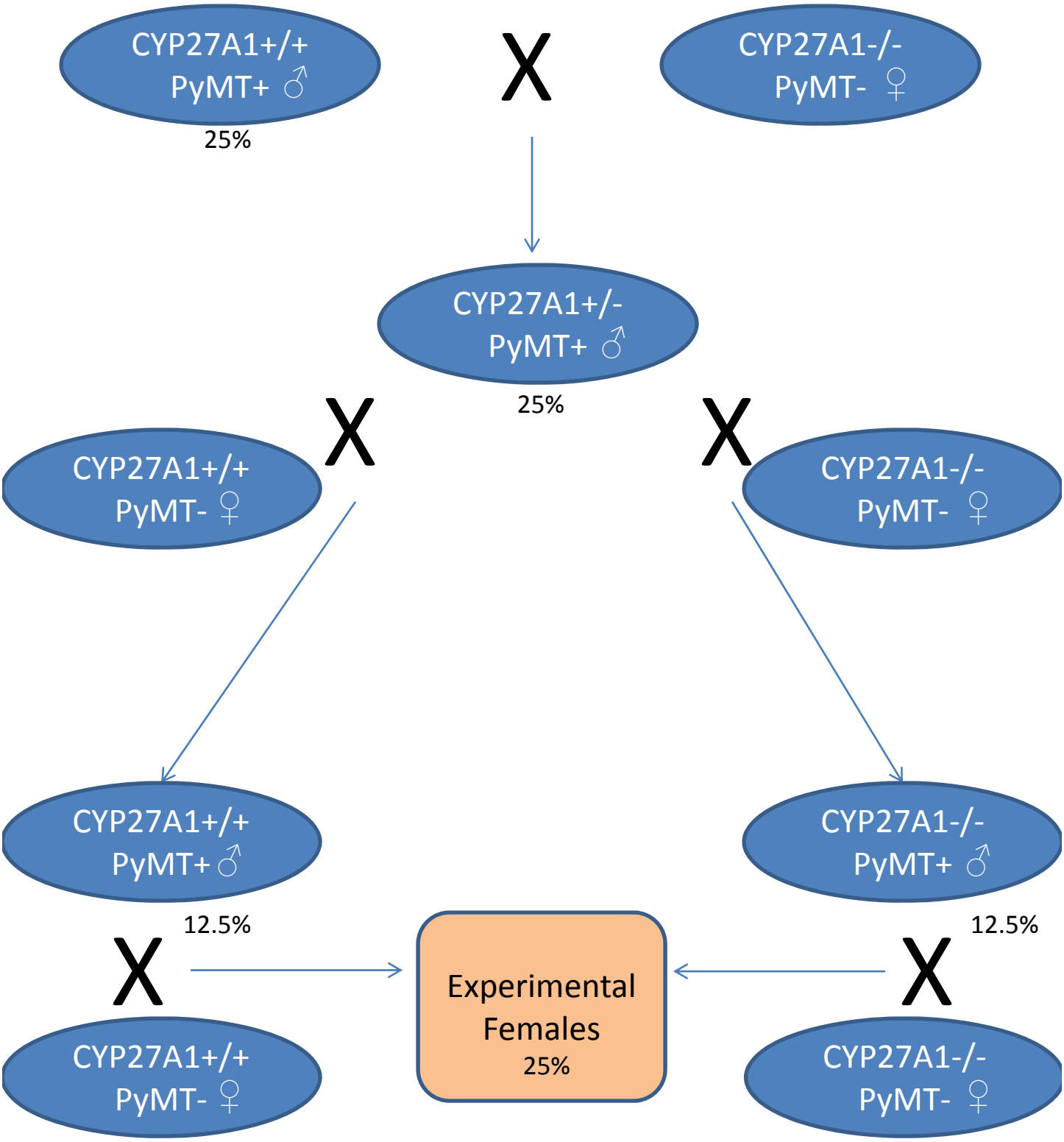

Figure 1: Breeding scheme to obtain PyMT positive mice that are either CYP27A1+/+ or CYP27A1-/-. A similar scheme is being used to generate CYP7B1+/+ and CYP7B1-/- mice positive for PyMT. Percentages represent the expected frequency of the given genotype resulting from the indicated cross.

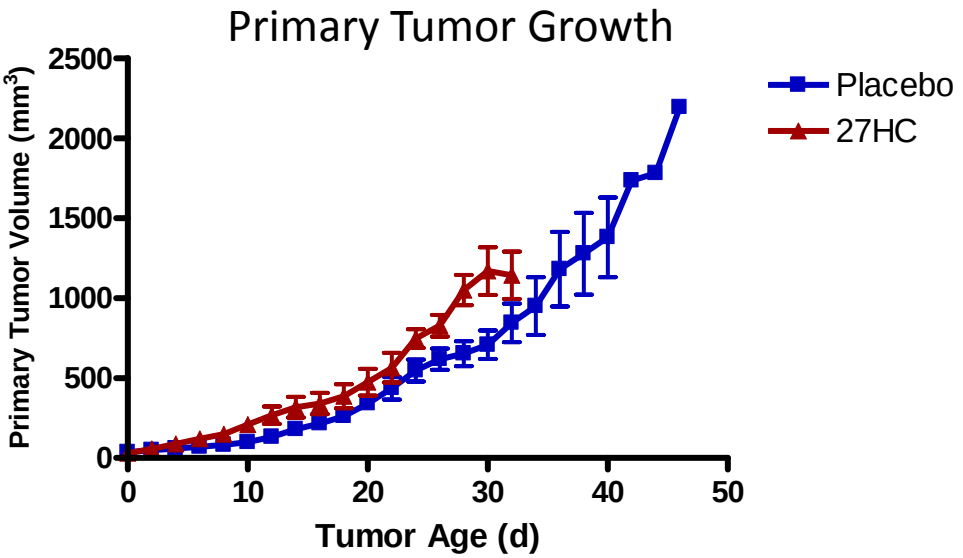

Figure 2: Effect of 27HC on primary tumor growth in PyMT transgene expressing mice. Mice were ovariectomized between 5 and 6 weeks of age. Upon detection of a palpable mammary tumor, mice were treated daily with subcutaneous injection of placebo or 27HC. Tumor growth was assessed by direct caliper measurement.

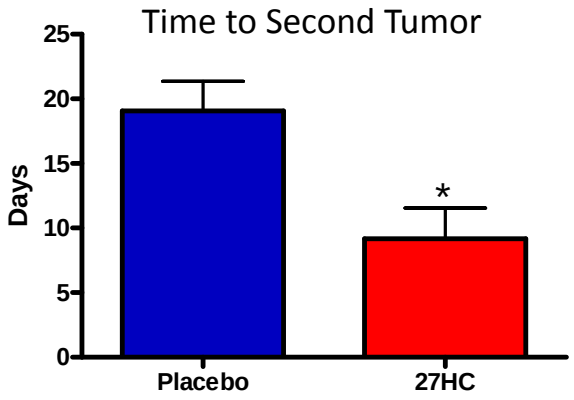

Figure 3: 27HC decreases the time between onset of primary tumor and detection of a secondary tumor by palpation. See Figure 2 for details. Star represents statistically significant difference (unpaired t-test, P<0.01).

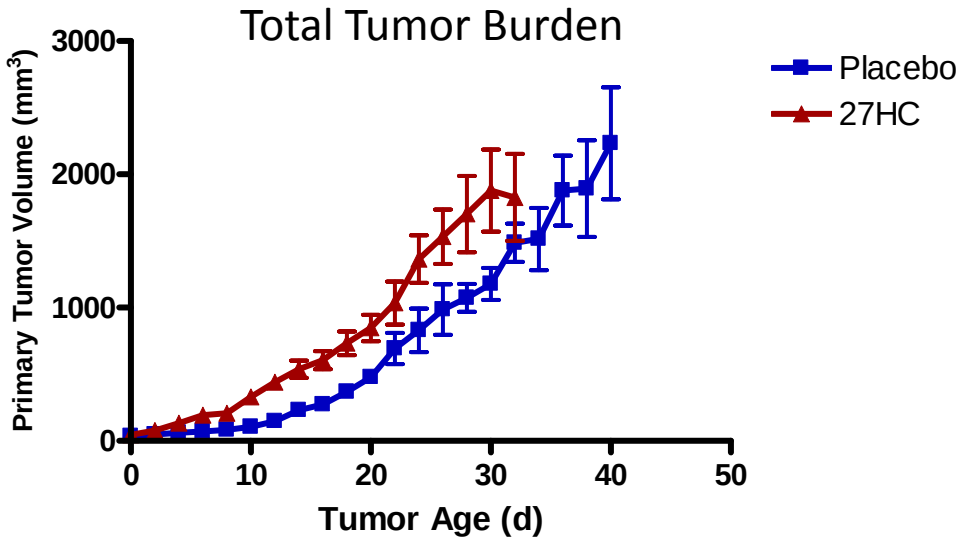

Figure 4: 27HC increases total tumor burden in PyMT mice. Total tumor burden was determined by adding the volumes of all individual tumors. See Figure 2 for details.

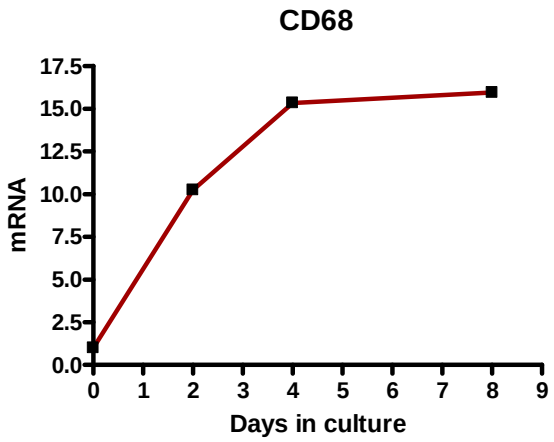

Figure 5: CD68 expression indicates positive differentiation of macrophages. Bone marrow extracts were cultured in the presence of M-CSF and harvested for RNA through time. CD68 expression was determined by quantitative PCR.

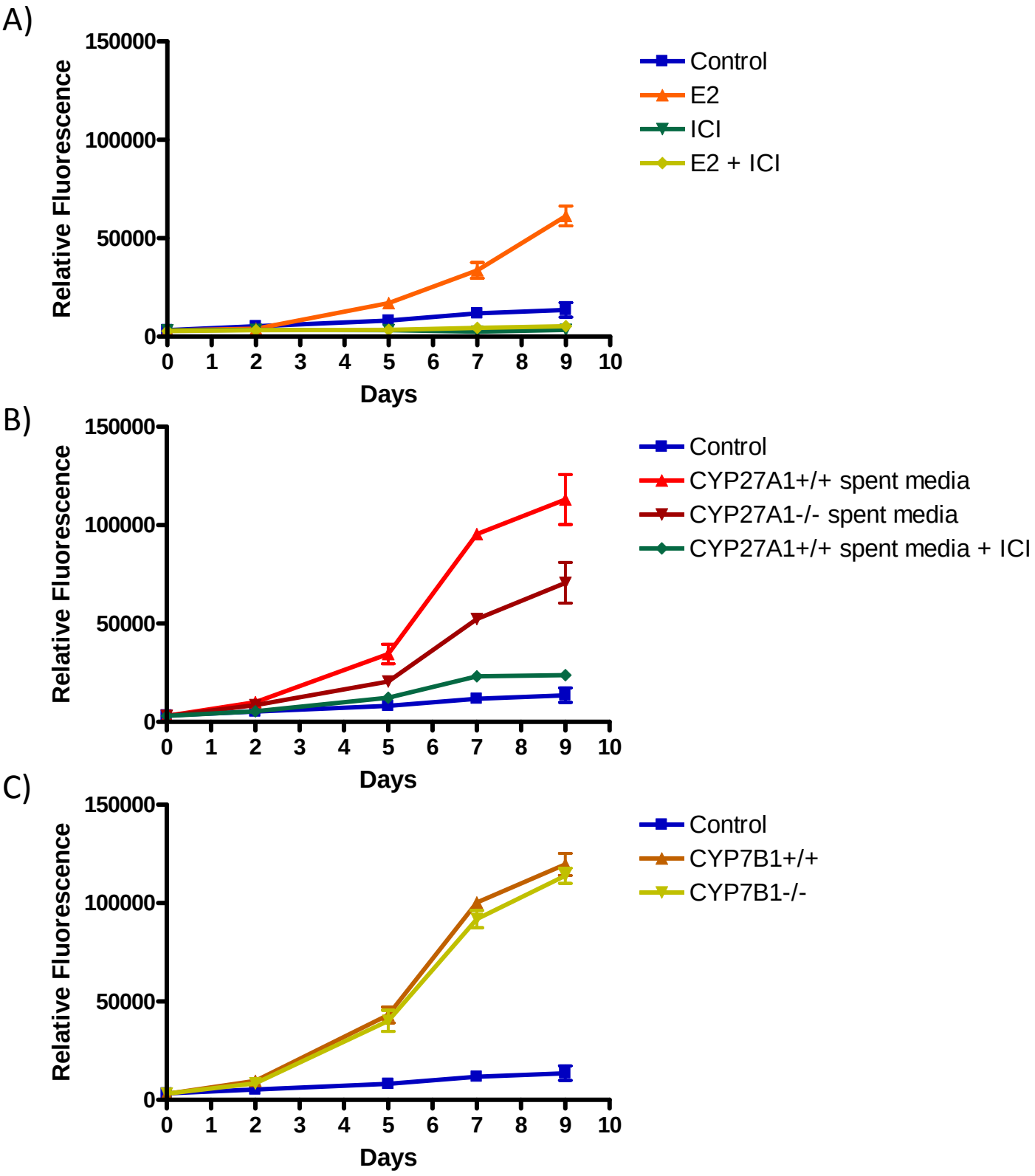

Figure 6: Effects of ER ligands and spent macrophage media on MCF7 cell proliferation. A) MCF7 cells were treated with DMSO (control) or indicated ligands in basal media. B/C) MCF7 cells were treated with DMSO in basal media (control) or a 50:50 mix of basal media and spent media from differentiated macrophages. Cell content was assessed by DNA content as measured by Hoescht fluorescence. All experiments were done side by side but have been displayed on separate graphs for clarity.

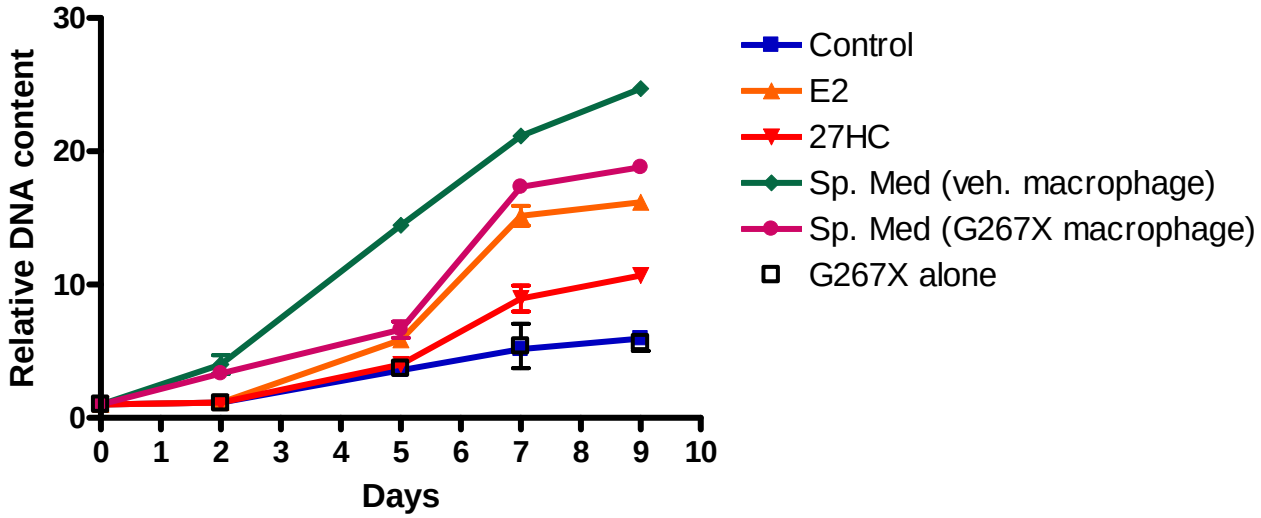

Figure 7: Preliminary data indicates that spent media from macrophages treated with an inhibitor of CYP27A1 (G267X) has a decreased stimulatory effect on proliferation. Control values represent MCF7 cells in basal media treated with vehicle alone. E2, 27HC and G267X alone represents cells treated in basal media. The other treatments were treated with spent media (Sp. Med) from macrophages treated with vehicle (veh.) or G267X.